Comparison of measuring energy metabolism by different (31) P-magnetic resonance spectroscopy techniques in resting, ischemic, and exercising muscle.

PRECLINICAL AND CLINICAL SPECTROSCOPY – Note

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Comparison of Measuring Energy Metabolism by Different ^{31}P-Magnetic Resonance Spectroscopy Techniques in Resting, Ischemic, and Exercising Muscle

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Alternate methods to quantify mitochondrial activity or function have been extensively used for studying insulin resistance and type 2 diabetes mellitus, namely saturation transfer and phosphocreatine (PCr) recovery. As these methods are in fact determining different parameters, this study aimed to compare saturation transfer results to PCr recovery measurements within the same group. Fifteen subjects underwent saturation transfer and ischemic exercise-recovery experiments. PCr decrease during ischemia (Q), induced by cuff inflation, served as an additional measure of resting ATP (adenosine triphosphate) production. ATP synthetic rate (fATP) measured by saturation transfer (0.234 ± 0.043 mM/s) was greater than (Q = 0.0077 ± 0.0011 mM/s), but correlated well with Q (r = 0.63 P = 0.013). Parameters of PCr recovery correlated well with fATP ($Q_{max,lin}$: r = 0.71, P = 0.003, $Q_{max,ADP}$: r = 0.66, P = 0.007) and Q ($Q_{max,lin}$: r = 0.92, P = 0.000002, $Q_{max,ADP}$: r = 0.76, P = 0.001). In conclusion, although saturation transfer yields higher ATP synthetic rates than PCr decrease during ischemia, their significant correlation indicates that fATP can be used as a marker of mitochondrial activity. The finding that both Q and fATP correlate with PCr recovery kinetics suggests that skeletal muscle with greater maximal aerobic ATP synthetic rates is also metabolically more active at rest. Magn Reson Med 67:898–905, 2012. © 2011 Wiley Periodials, Inc.

Key words: mitochondria; skeletal muscle; magnetization transfer; exercise recovery

INTRODUCTION

Skeletal muscle is the main contributor to energy expenditure and an important target of insulin, which stimulates myocellular nutrient uptake and storage. In this context, the majority of glucose uptake and storage as glycogen during stimulation by insulin occurs in skeletal muscle (1). Impaired insulin sensitivity, also termed insulin resistance, not only comprises reduced glucose uptake, but has also been associated with abnormalities of mitochondrial content, distribution and function as assessed in vitro from muscle biopsies (2).

^{31}P-magnetic resonance spectroscopy has proven to be a potent tool for investigating energy metabolism in vivo (3). Recently, several studies reported skeletal muscle ATP production using the ^{31}P-magnetic resonance spectroscopy-based saturation transfer technique (4–6). Lower ATP turnover was found in nondiabetic first-degree relatives of patients with type 2 diabetes mellitus (T2DM) (4) and in patients with overt T2DM (5) along with insulin resistance. In general, the quantity measured by saturation transfer was referred to as a marker of mitochondrial fitness (6), activity, or function (4).

An alternate ^{31}P-magnetic resonance spectroscopy technique, based on exercise recovery experiments, has been used extensively to investigate mitochondrial function in various phenotypes (7,8) and more recently also in insulin resistance and T2DM (9,10). Purely aerobic, mixed, or anaerobic exercise is used to deplete PCr. PCr recovery relies almost exclusively on aerobic metabolism (7) and is therefore likely reflecting maximal mitochondrial output or mitochondrial capacity (8). Of note, prolonged PCr recovery has been associated with elevated plasma glucose concentrations (9,10) and insulin resistance (11) in some, (9,10) but not all reports (12).

In PCr recovery measurements, it is assumed that PCr kinetics reflects ATP producing pathways, coupled by the creatine kinase reactions. Saturation transfer measurements determine the synthesis rate of ATP more directly. As it is generally assumed that ATP production is demand-driven, determining the rate of ATP synthesis in the resting state (as is typically done by saturation transfer measurements) probably gives information about mitochondrial "activity" rather than mitochondrial "capacity."

Although saturation transfer ATP production rates and PCr recovery kinetics are both used to estimate in vivo mitochondrial function, different parameters are

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measured. Thus, we aimed to compare the two measures and to test their interrelation. Furthermore, metabolic activity of the muscle was also determined by the rate of ischemia-induced PCr decrease, permitting direct comparison of two independent measures of resting state ATP turnover. The rate of ischemia-induced PCr decrease was compared with ATP synthetic rate, measured by saturation transfer and to maximal aerobic ATP synthetic rate as calculated from PCr recovery kinetics. As post-exercise metabolic activity could be different from the pre-exercise state (13), we also studied whether ischemia-exercise-recovery affects saturation-transfer ATP synthesis.

METHODS

Nineteen nonobese male volunteers were studied, of whom 15 were included in the analysis (age 27.6 ± 6.1 years, body mass index 21.9 ± 1.2 kg/m^2 fasting plasma glucose 85 ± 6 mg/dL, 73–94 mg/dL, triglycerides 106 ± 56 mg/dL, 67–254 mg/dL). The inclusion criteria were: male sex, age between 18 and 45 years, body mass index between 18 and 25 kg/m^2, nonsmoking status for at least 3 months, and normal medical history and examination. Exclusion criteria were any of the following: regular medication, drug treatment in the previous 3 weeks including over-the-counter drugs, alcohol abuse, participation in a clinical trial during the 3 weeks preceding the study, hypertension, dysglycemia, dyslipidemia, symptoms of any disease during the 2 weeks before the study day, specific history of urolithiasis, gastrointestinal, liver or kidney disease, blood donation during the previous 3 weeks, glucose-6-phosphate dehydrogenase deficiency, thalassemia, haemochromatosis; any not removable metallic or paramagnetic device or claustrophobia. Subjects were studied on a 3-T MR scanner (Bruker, Ettlingen, Germany) using a 10-cm ^{1}H/^{31}P surface coil positioned below the subjects' calf. The sensitive volume of the coil covered the gastrocnemius and soleus muscles. Two volunteers felt uncomfortable within the magnet and were unwilling to complete the experiment according to the inclusion/exclusion criteria, and one volunteer had to be excluded because of intensive physical activity before the experiment. One data set could not be used due to an error in signal recording.

Experimental Protocol

The experiment consisted of three blocks: saturation transfer experiment, ischemia–recovery, and another saturation transfer experiment. The setup is depicted in Fig. 1. The study protocol was in line with the most recent version of the Declaration of Helsinki (2008) and approved by the appropriate institutional ethical board. Informed consent was obtained from each volunteer after explanation of the purpose, nature and potential complications of the study.

Saturation Transfer Experiment

The chemical exchange between Pi and ATP was measured using the saturation transfer technique (14,15). All scans were based on a pulse-acquire experiment: T_R was

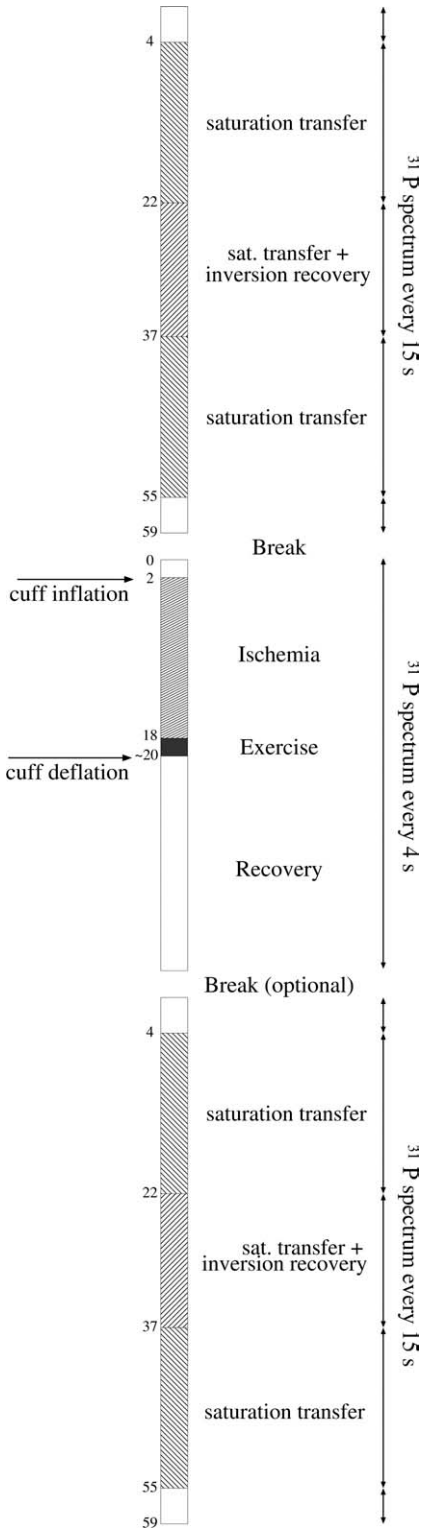

FIG. 1. The experiment consisted of three parts, two saturation-transfer experiments and a ischemia reperfusion experiment. Time from top to bottom in minutes.

15 s, a nonselective 90° excitation pulse followed by 10-kHz bandwidth 200 ms data acquisition. The γ-ATP resonance was saturated using continuous irradiation during the recovery period of the sequence (72 acquisitions). The results were compared with a control experiment,

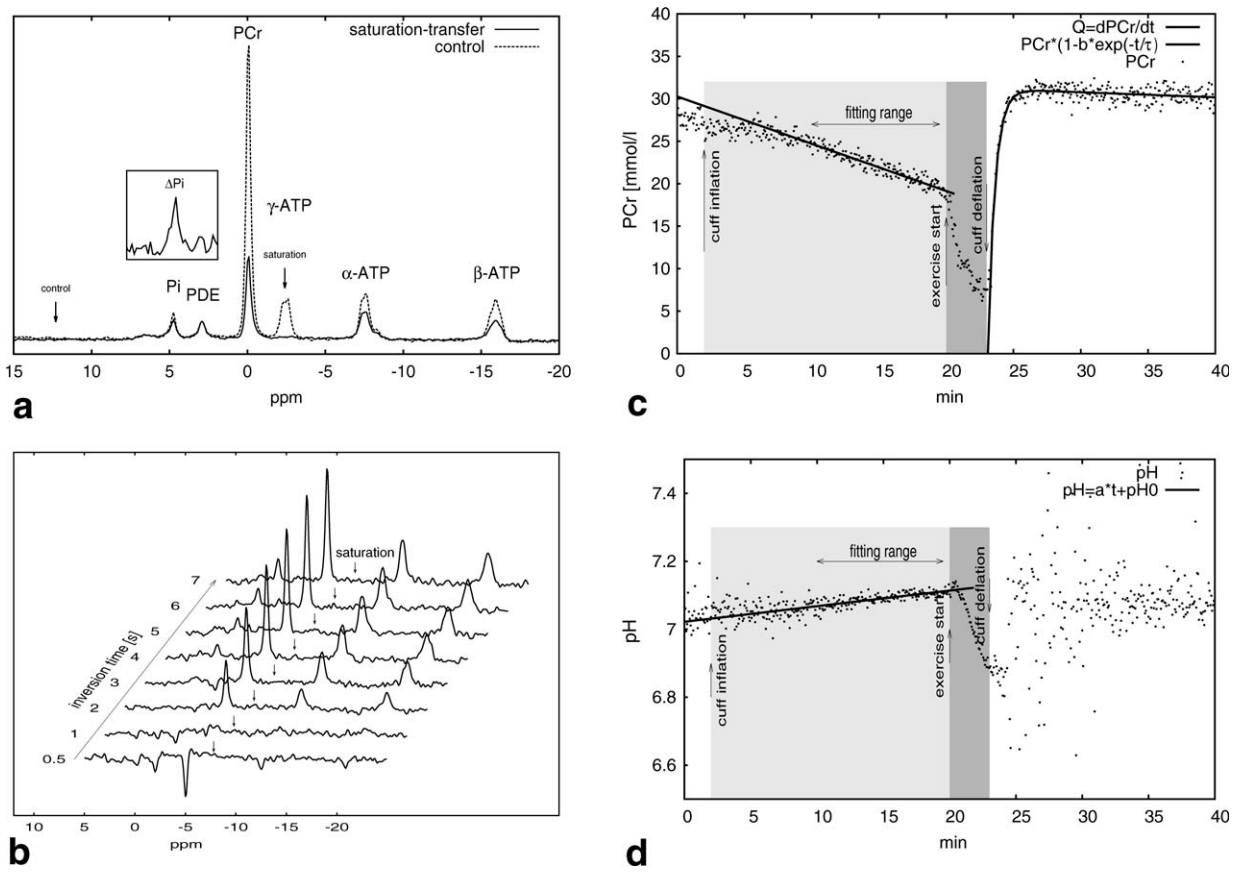

FIG. 2. **a**: Two ^{31}P spectra, showing the saturation transfer effect on Pi (magnified difference spectrum) Arrows indicate the saturation frequencies (γ-ATP and mirrored about Pi for the control scan). **b**: Inversion-recovery measurement for $T_{1,app}$. **c**: Time course of PCr during ischemia (light grey), exercise (grey), and reperfusion. The solid lines indicate the linear fit (Q) and the exponential fit (τ_{PCr}). **d**: Time course of pH during ischemia (light grey), exercise (grey) and reperfusion The solid line shows the linear fit during ischemia for calculating L and F. Pi is barely quantifiable in the recovery phase, therefore pH values are widely scattered. Data for all graphs are from the same subject.

mirroring the saturation frequency about Pi, for the Pi → ATP reaction (48 acquisitions), see Fig. 2a. Apparent T_1 was also measured by inversion recovery (adiabatic nonselective inversion) while saturating γ-ATP (eight inversion times between 0.5 s and 7 s; six averages), see Fig. 2b. The different number of acquisitions are the result of an interleaved acquisition scheme to optimally use the scan time, i.e., more averages for the lower signal-to-noise-ratio (SNR) scans with saturation, less for higher SNR control scans.

Spectra were processed using the XWIN-NMR software (Bruker, Ettlingen, Germany). For apparent T_1 and saturation transfer, the signals were quantified using signal amplitude after manually adjusting phase and setting the baseline. In case where the metabolite concentrations were quantified, peaks were integrated.

Ischemia–Recovery experiment

Heart rate (66 ± 8, 52–75 beats/min), systolic (123 ± 11, 108–147 mmHg) and diastolic blood pressure (69 ± 13, 43–83 mmHg) were monitored before scanning the volunteers. ^{31}P spectra were acquired every 4 s (pulse-acquire experiment, otherwise similar to the baseline scans used in the saturation transfer block). After 2 min, the cuff was inflated to 200 mmHg and remained so for about 20 min. Eighteen minutes within ischemia, subjects were instructed to do plantar flexions during the last, approximately two, minutes of ischemia until exhaustion on a custom built nonmagnetic exercise rig (16). PCr (Fig. 2c) and Pi resonances as well as pH (Fig. 2d) were quantified using AMARES (17) in jMRUI (18).

Calculations

For the absolute quantification an ATP concentration of 8.2 mM cellular water was assumed (19).

Saturation Transfer ATP Synthetic Rate

The forward rate constant k was calculated from the ratio of saturation (M_s) and control experiments (M_0) (Fig. 2A) and the apparent longitudinal relaxation time $T_{1,app}$ (Fig. 2b).

$$k = \frac{1}{T_{1,app}}\left(1 - \frac{M_s}{M_0}\right).$$

Multiplying by the Pi concentrations obtained from a separate scan yielded the exchange rates fATP = kPi. Values for fATP determined before and after ischemic

Table 1
Results of the Saturation Transfer and Ischemia-Exercise-Recovery Experiments

	Mean ± standard deviation
Resting state (mean of pre- and post-ischemic values	
Pi [mM]	3.84 ± 0.40
k (Pi → ATP) [1/s]	0.061 ± 0.011
fATP [mM/s]	0.234 ± 0.043
PCr [mM]	25.16 ± 3.91
Pi/PCr	0.155 ± 0.029
Ischemic resting state	
Net aerobic ATP turnover *Q* [mM/s])	0.0077 ± 0.0011
Slope of pH, dpH/dt [1/s]	0.000075 ± 0.000011
Glycolytic ATP production *L* [mM/s]	0.00077 ± 0.00056
Total ATP turnover *F* [mM/s]	0.0085 ± 0.0016
Recovery	
Initial PCr recovery rate (V_{PCr}) [mM/s]	0.44 ± 0.14
PCr time constant of recovery (τ_{PCr}) [s]	34.7 ± 5.8
End-exercise pH	6.94 ± 0.12
Change in PCr during recovery ΔPCr [mM]	14.84 ± 4.20
$Q_{max,ADP}$ [mM/s]	0.59 ± 0.18
$Q_{max,lin}$ [mM/s]	0.74 ± 0.18

exercise were averaged and these average values were used for comparison of fATP with other parameters.

Ischemic PCr Decrease

A linear fit of the PCr signal (Fig. 2c) was used to calculate $Q = \delta PCr/\delta t$ based on (20). The anaerobic contribution to net resting ATP turnover L was estimated using pH variation according to Kemp and Radda (8), assuming no efflux of protons in the ischemic state, using the equation:

$$L = \frac{3}{2}\left(\varphi\frac{\delta PCr}{\delta t} - \beta\frac{\delta pH}{\delta t}\right).$$

The pH was calculated from PCr and Pi resonance frequencies (21), δpH/δt is a linear fit (Fig. 2d). The calculations were performed assuming a fixed buffer capacity $\beta = 30$ slykes and stochiometry factor $\phi = 0.36$. The sum of the net aerobic and net anaerobic ATP synthesis rate results in total resting net ATP synthesis rate (F).

$$F = L + Q = \frac{3}{2}\left(\varphi\frac{\delta PCr}{\delta t} - \beta\frac{\delta pH}{\delta t}\right) + \frac{\delta PCr}{\delta t}$$

PCr Recovery and Maximal Aerobic ATP Synthetic Rate

Post-exercise post-ischemic PCr recovery were fitted to a single exponential equation plus a linear component to account for long-term instabilities. Data from cuff release until the end of the experiment were used (about 15 min), resulting in more than 200 single data points per subject. A nonlinear least square fit was used to model the PCr data in perl/PDL (http://pdl.perl.org) (22) using the Fit-Levmar module. From the exponential term $PCr(t) = \left[PCr|_{\text{post-recovery}}\left(1 - b \cdot e^{-t/k}\right)\right]$, the rate constant k was determined and from this, $\tau_{PCr} = 1/k$ (Fig. 2c). The maximal oxidative flux (Q_{max}) was also calculated according to the ADP based model (23) and according to the linear model (24).

$$Q_{\max -ADP} = V_{PCr} \cdot \left(1 + \frac{K_m}{ADP|_{\text{end-exercise}}}\right)$$

$$Q_{\max -lin} = \frac{1}{\tau_{PCr}} \cdot PCr|_{\text{post-recovery}}$$

The initial PCr recovery rate (V_{PCr}) roughly represents ATP turnover at the end of exercise and was calculated as follows:

$$V_{PCr} = \frac{1}{\tau_{PCr}}\Delta PCr|_{\text{recovery}}$$

ADP concentrations were calculated according to the model of Lawson and Veech (25) assuming that the creatine kinase reaction is at equilibrium and a K_m of 30 μM (19). Furthermore, it was assumed that 15% of total creatine was not phosphorylated in the resting state.

$$ADP = \frac{ATP \cdot Cr}{PCr \cdot H^+ \cdot K_{eq}}$$

Statistics

Results are presented as mean ± standard deviation. The relationship between parameters was assessed by Pearson correlation coefficient using SPSS for Windows 16.0 software (SPSS Inc., Chicago, IL). The paired student's *t*-test was used to compare pre- and post-exercise values. Results were considered significant at P values <0.05.

RESULTS

Saturation Transfer

Mean fATP was 0.22 ± 0.05 mM/s before the ischemic exercise protocol. Similar rates were measured afterwards (0.24 ± 0.05 mM/s, $P = 0.31$). The averaged pre- and post exercise values were 0.23 ± 0.04 mM/s (Table 1). Pi concentrations were higher (3.65 ± 0.48 mM vs. 4.04 ± 0.50 mM, $P = 0.020$) after the ischemic

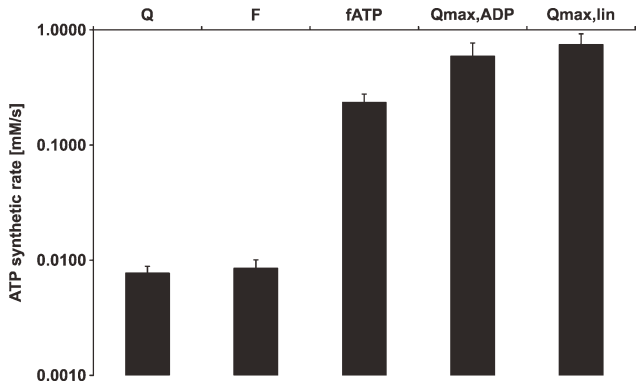

FIG. 3. Log-scale bar plot of different measures of ATP synthesis from left to right: net aerobic ATP synthesis ($Q = \delta PCr/\delta t$); total net ATP turnover during ischemia ($F = Q + L$); fATP measured by saturation transfer; maximum oxidative ATP fluxes $Q_{max,ADP}$ and $Q_{max,lin}$

exercise protocol, also the ratio of Pi to PCr concentrations was higher after ischemic exercise (initially 0.148 ± 0.031, vs. 0.163 ± 0.029, $P = 0.002$).

PCr Decrease and Anaerobic ATP Production During Ischemia

After several minutes of cuff inflation, PCr started to decrease approximately linearly from resting values (Table 1) to 19.0 ± 2.9 mM, while pH increased slowly and steadily until the onset of exercise. The linear decrease in PCr was apparent after 8 min during inflation in all subjects. The slope of PCr Q was 0.0077 ± 0.0011 mM/s. When taking into account the contribution of anaerobic glycolysis (L), total resting net ATP turnover (F) was 0.0085 ± 0.0016 mM/s. Results from one volunteer are given in Fig. 2c+d).

PCr Recovery After Exercise

During exercise, PCr concentration decreased rapidly from 19.0 ± 2.9 mM, reaching 10.6 ± 3.6 mM at the end of exercise. pH decreased slightly during exercise, however stayed above 6.7 in all cases (mean end-exercise pH: 6.94 ± 0.12). PCr concentration increased rapidly after cessation of exercise and followed a roughly monoexponential time course. The values for initial PCr recovery rate (V_{PCr}) and time constant (τ_{PCr}) are given in Table 1, also the maximal aerobic flux, calculated according to the ADP-based model ($Q_{max,ADP}$) and the linear model ($Q_{max,lin}$) are given. In Fig. 3, all the different measures of ATP synthesis are compared.

Correlation Analyses

The rate of saturation transfer significantly correlated with parameters of PCr recovery: fATP linearly correlated to τ_{PCr} (slope: −89 ± 27 mM/s^2, intercept: 55.5 ± 6.5 mM/s, $r = -0.67$, $P = 0.006$), $Q_{max,lin}$ (slope: 2.93 ± 0.80, intercept: 0.06 ± 0.19 mM/s, $r = 0.71$, $P = 0.003$) and $Q_{max,ADP}$ (slope: 2.71 ± 0.85, intercept: 0.04 ± 0.20 mM/s, $r = 0.66$, $P = 0.007$). Q (slope 0.016 ± 0.006 intercept 0.0039 ± 0.0013 mM/s, $r = 0.63$, $P = 0.013$) as well as F (slope 0.019 ± 0.008 intercept 0.0040 ± 0.0020 mM/s $r = 0.53$, $P = 0.042$) also correlated with fATP.

Further, the parameters of PCr recovery correlated with measures of resting ATP turnover, based on PCr decrease: Q correlated with τ_{PCr}, $Q_{max\text{-}lin}$ and $Q_{max\text{-}ADP}$ (slope: −3846 ± 936 mM/s^2, intercept: 64 ± 7 mM/s, $r = -0.75$, $P = 0.001$; slope: 145 ± 18, intercept: −0.38 ± 0.18, mM/s, $r = 0.92$, $P = 0.000002$; and slope: 119 ± 29, intercept: −0.30 ± 0.22 mM/s, $r = 0.76$, $P = 0.001$) respectively. τ_{PCr}, $Q_{max\text{-}lin}$ and $Q_{max\text{-}ADP}$ correlated also with F (slope: 2480 ± 769 mM/s^2, intercept: 56 ± 7 mM/s, $r = -0.67$ $P = 0.006$; slope: 97 ± 17, intercept: 0.08 ± 0.15 mM/s, $r = 0.84$, $P = 0.00008$ and slope: 79 ± 22, intercept: −0.07 ± 0.19 mM/s, $r = 0.70$, $P = 0.004$, respectively).

Body mass index correlated with Q ($r = 0.78$, $P = 0.0009$), $Q_{max,lin}$ ($r = 0.67$, $P = 0.006$), $Q_{max,ADP}$ ($r = 0.63$, $P = 0.012$), L ($r = 0.61$, $P = 0.015$), and F ($r = 0.78$, $P = 0.0006$).

DISCUSSION

We compared three methods to examine oxidative metabolism in skeletal muscle: saturation transfer measurements, PCr decrease during ischemia and PCr recovery after exercise. Each method is based on certain assumptions and therefore only provides an estimate of oxidative metabolism. Although we clearly show that the parameters of ATP synthesis determined with these three methods correlate well (Fig. 4), absolute values differed substantially (Fig. 3). Saturation transfer measurements revealed more than 20-fold higher ATP production rates than estimation based on ischemia-induced PCr decrease. A meta-analysis of previous publications (26) reported also much higher values for saturation transfer than for other measurements of resting ATP turnover. To our knowledge, a direct comparison of saturation transfer with the other methods has not been reported before in one single group of humans.

The results of PCr recovery are assumed to reflect maximal mitochondrial capacity to synthesize ATP (8). It is generally assumed that ATP production in skeletal muscle is demand-driven (8), implicating that any measurement of resting ATP turnover mirrors resting energy need. Both estimates of resting ATP turnover used in the current study find a considerable range, which indicates individual differences in resting metabolic rate of the muscles of the lower leg. Differences in training status, age or body mass index could also underlie this finding. It is beyond the scope of this study to determine this. The range of the fluxes is well in line with other studies in other subjects (27). As energy demand in the resting state is not maximal, measurements performed at rest rather assess mitochondrial "activity" than maximal "capacity." On the other hand, diminished mitochondrial activity has been suggested to indicate mitochondrial "dysfunction" (28). If this is valid, ATP synthesis measured by saturation transfer should correlate with parameters of mitochondrial capacity, e.g., PCr recovery kinetics.

Here we report a significant albeit not tight correlation between fATP and Q_{max}. This suggests a relationship between resting and maximal mitochondrial function.

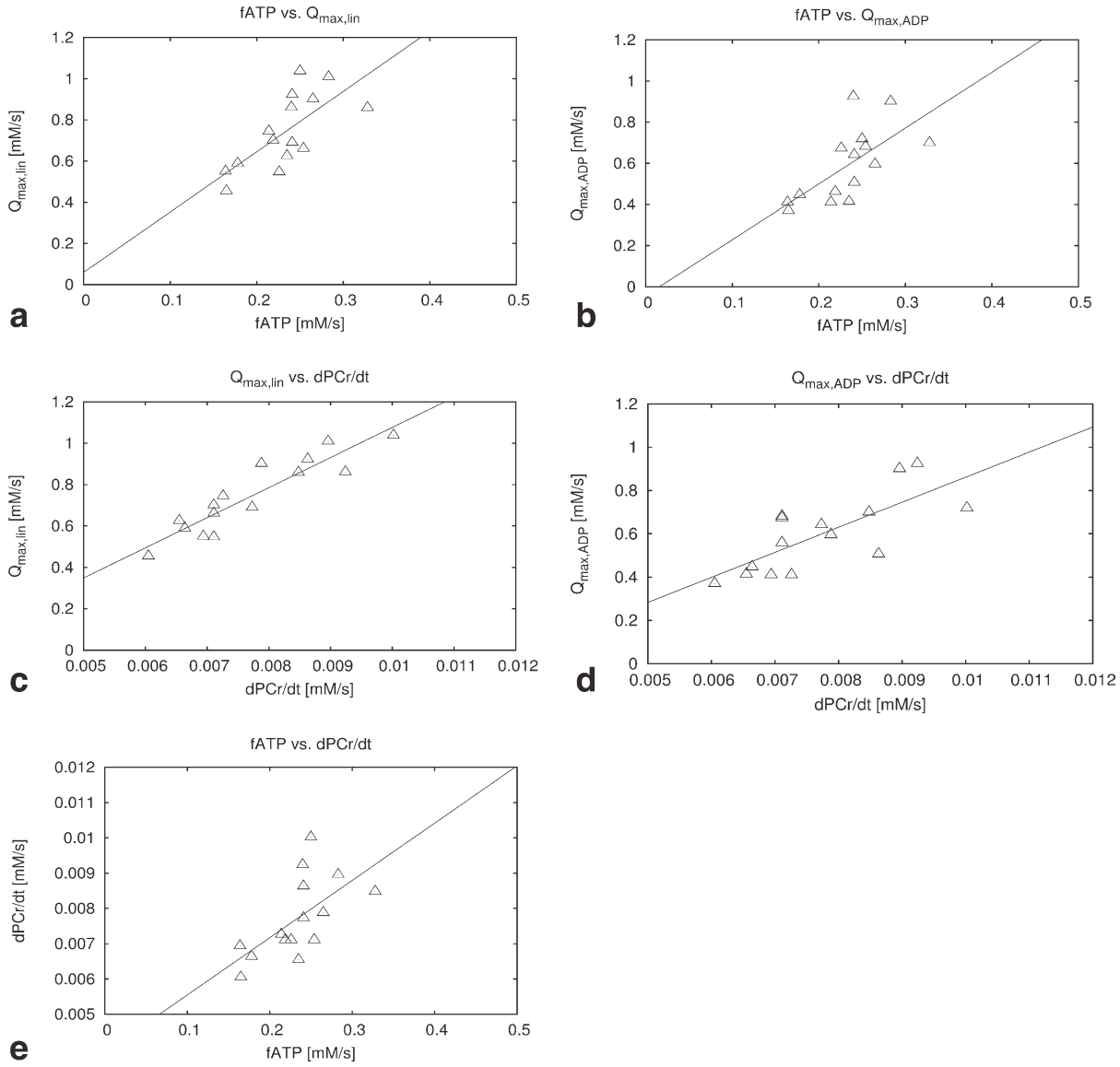

FIG. 4. Plots showing correlations of saturation transfer rates with (**a**) $Q_{max,lin}$ (**b**) with $Q_{max,ADP}$ (**e**) with δPCr/δt c) δPCr/δt with $Q_{max,lin}$ and (**d**) with $Q_{max,ADP}$

The underlying mechanisms for this observation remain uncertain. Likewise, we find a strong correlation between the parameters of PCr recovery and the alternate measurement of resting mitochondrial activity, the rate of PCr decrease during cuff-ischemia. In the absence of oxygen, the rate of PCr breakdown compensates for lacking oxidative phosphorylation and has been suggested as a measure of net aerobic ATP turnover in the resting state (8). As to the relationship between resting activity and maximal capacity, it is of note that oxygen consumption and ATP need have been shown to be diminished in skeletal muscle of elderly subjects (20), known to have lower mitochondrial function (29). These data are in line with the present findings of lower ATP synthesis in humans with delayed PCr recovery kinetics. Furthermore, resting ATP synthetic rate has also been shown to correlate with maximal whole body oxygen uptake (VO_{2max}) (30), a parameter of whole body mitochondrial fitness, again pointing to a relation between energy need of skeletal muscle and oxidative capacity. The molecular basis of this relationship is yet unknown and needs further investigation. The correlation of fATP with PCr recovery kinetics seems in contrast to earlier animal studies using blockers of complex I of the respiratory chain, showing that PCr recovery kinetics and fATP were affected differentially (31).

The higher values of ATP synthetic rates, determined by saturation transfer measurements compared to the other methods are in line with previous reports summarized by Kemp (26). Glycolysis, which could contribute to fATP, is assumed to operate at low rates in the resting state (20). Although net glycolytic ATP production cannot explain the higher values found with saturation transfer measurements, glycolytic ATP forming reaction might still play an important role. One should be aware that saturation transfer is determining unidirectional

fluxes. Therefore, if reactions are operating close to equilibrium, the unidirectional flux can be much higher than the net flux. Therefore, the most likely explanation for the overestimation of the rate of ATP synthesis by saturation transfer is that certain ATP forming reactions in resting skeletal muscle are operating relatively close to equilibrium. There is evidence from yeast that glycolytic reactions contribute strongly to fATP measured by ^{31}P-magnetic resonance spectroscopy (32,33), indicating that reversible glycolytic reactions might explain the higher ATP synthetic rates. Finally, mitochondrial ATP formation is not strictly irreversible (34). As a consequence, the apparent forward flux (fATP) exceeds the net production many times (26). Nevertheless, our data show that this parameter seems to be linearly related to an alternate measure of resting ATP turnover (PCr decrease). However, deviations in slope and intercept from one and zero indicate that fATP and Q measure different parameters respectively. Nevertheless, fATP is a marker of resting ATP synthetic rate, as higher net ATP flux also mirrors increased fATP flux by saturation transfer. Furthermore, fATP has been repeatedly shown to be responsive to insulin stimulation, a condition known to increase energy expenditure. The insulin-induced increase in fATP is pronounced in insulin sensitive, but poor or absent insulin resistant states (5,30), again paralleling the response in energy expenditure. This again argues for fATP measurements as markers of oxidative activity.

As expected, the ATP production rate immediately after exercise (estimated form the initial rate of PCr recovery) is higher than the ATP production rates in the resting state (measured by decrease of PCr or saturation transfer), reflecting exercise-induced stimulation of ATP synthesis. It should be noted that the initial rate of PCr recovery immediately after exercise (V_{PCr}) depends on ATP turnover during exercise, and therefore on exercise intensity. τ_{PCr}, (or the half-time or rate constant) can be used directly as parameter of mitochondrial function, as it is assumed to be independent of exercise intensity, as long as no substantial acidification occurs. With the current protocol, the end-exercise pH stayed relatively high (above 6.7 in all subjects), justifying the use of the time-constant τ_{PCr} as a measure of mitochondrial capacity and the deduced calculations of Q_{max}.

Our data and those from earlier reports (20) indicate that the rate of the decrease of PCr during ischemia is an alternate option to saturation transfer measurements to quantify resting aerobic mitochondrial activity. However, the frequently painful ischemia will not be generally tolerated by subjects and can be hazardous particularly in patients with vascular diseases. In that respect, it is important to use cuff pressure well above the systolic value, as residual blood flow could affect PCr decrease, thereby underestimating ATP synthesis.

For the calculation of anaerobic glycolysis, some assumptions on buffer capacity and reaction stochiometry are necessary which affect outcomes regarding the anaerobic component of resting ATP turnover, as well as the total ATP turnover. Importantly however, the decrease in PCr, which is interpreted as equivalent to resting oxidative ATP turnover, can be determined directly and is not depending on additional parameters.

In conclusion, this study shows that (i) mitochondrial capacity, as determined by PCr recovery kinetics, correlates with resting mitochondrial activity as measured by PCr depletion and by saturation transfer and (ii) ATP synthetic rates measured by saturation transfer yields greater values than measured by ischemic decrease of PCr.

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